Functionalization of Polycaprolactone via Aminolysis for Optimal Oxygen Sensor Biodegradability

Research Thesis

Presented in partial fulfillment of the requirements for graduation *with research distinction* in the undergraduate colleges of The Ohio State University

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Abstract

A primary amine was utilized to react with and chemically modify a specific polyester, polycaprolactone (PCL), enabling biomedical sensing functions along with additional biological utility[1]. PCL is a good substrate for reaction chemistry exploration as it has a role in a range of biomedical applications due to its relatively slow biodegradability[2]. While PCL modifications have been undertaken previously using a variety of approaches, much of the resulting evaluation is based on performance in the application, rather than definitive confirmation that chemical attachment has truly taken place[3]. True verification of these chemical modifications is absent from the literature. The work described here addresses this central challenge addressed here via the use of HMBC NMR combined with XPS and UV-VIS analysis.

Acknowledgments

I would like to offer my sincerest gratitude to Dr. Lannutti, my technical advisor, for allowing my chemistry background to thrive in his material science and engineering research group. My appreciation also goes out to Daniel Cybyk and Bonnie Reinsch, graduate students who are a part of Dr. Lannutti's research team, who I reported to and had tremendous amount of patience with me. I would like to thank Tanya Whitmer for showing endless amount of kindness and guiding me through the various NMR techniques central to this investigation. Lastly, I would like to thank Dr. Terry Gustafson and Dr. Mike Chang for networking between the Chemistry department where much of my research took place and the Material Science and Engineering.

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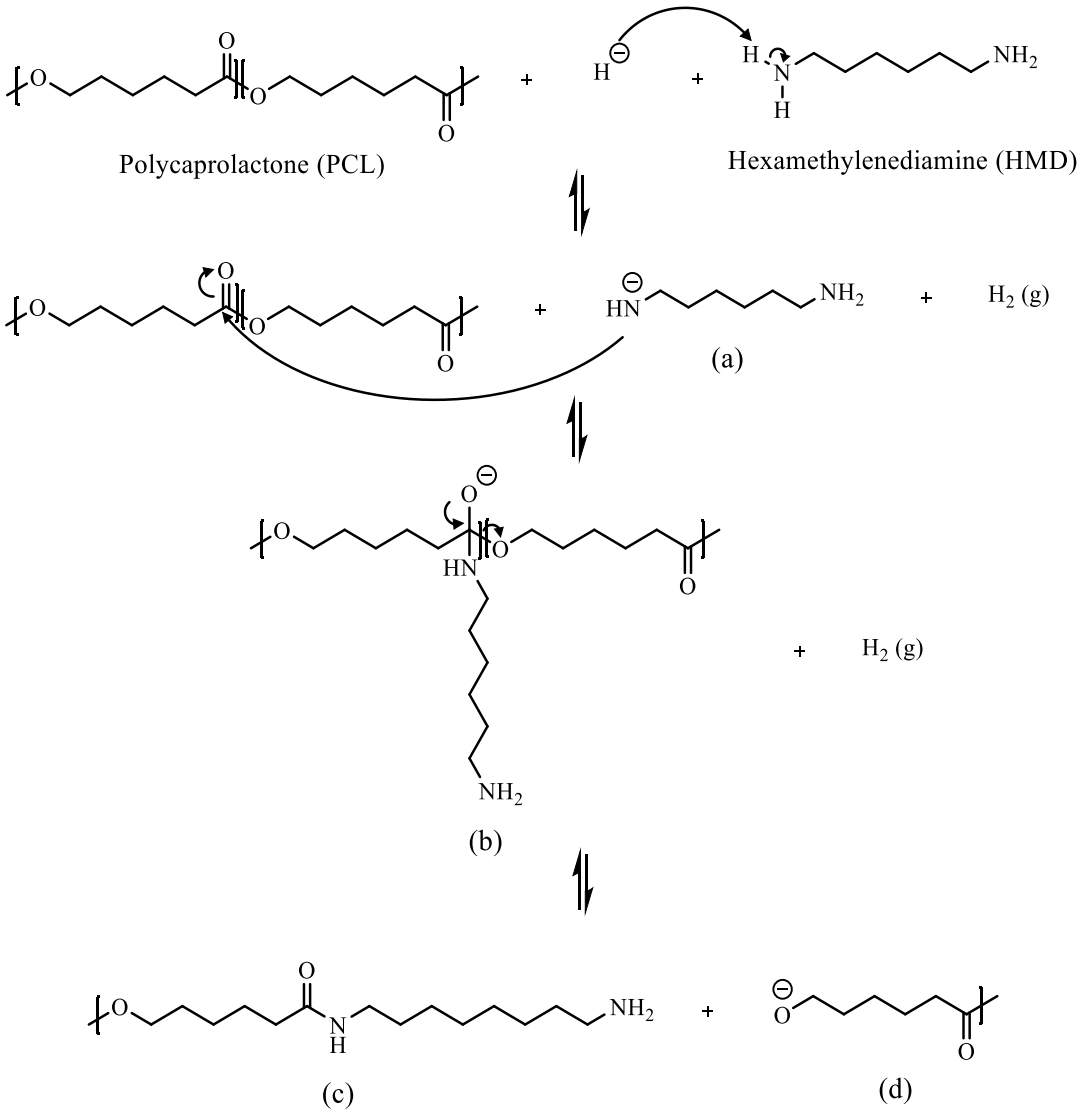
Introduction

Polycaprolactone (PCL) is mainly known for its role as a useful medical plastic due to its slow rate of biodegradation[4]. When implanted into the human body, this polymer degrades through hydrolysis of its ester linkages[5]. In the desired application, a series of clinical applications pertain to its use in creating *in vivo* sensors — specifically, oxygen sensors[6]. PCL displays an initial resistance to water and is therefore considered hydrophobic; its surface slowly undergoes chemical modification, gradually becoming hydrophilic and hence more biocompatible. However, while PCL has considerable biomedical utility, it also suffers from specific drawbacks. A lack of information on the kinetics of biodegradation is a notable factor limiting greater application in tissue engineering[7]. In addition, beyond its mechanical role this polyester has no specific chemical functionality unless chemically modified.

Changes to the functionality of a variety of polymer surfaces can be achieved via plasma modification, a phenomenon which is gradually reversed over time[8]. Other methods such as UV irradiation have also been explored[9]. However, aminolysis is a widely-utilized method designed to functionalize polymeric surfaces via amine-group attachment. Such positively-charged PCL provides a more functional surface by enabling much better cell adhesion[10]. Furthermore, the utilization of wet chemical methods could enable even more efficient 'tailoring' of biological performance.

Scheme 1 shown below details the chosen reaction mechanism between polymer PCL chains and hexamethylenediamine (HMD), the reactant used to initiate functionalization of the biomaterial. The addition of sodium hydride serves an important role as a catalyst that increases HMD reactivity in solution due to its initial deprotonation. Sodium hydride is commonly used for deprotonation of polyesters and other functional groups enabling promotion of nucleophilic substitution. In PCL, an acyl substitution takes place in which the deprotonated amine end group (a) acts as a nucleophile, forming an unstable tetrahedral intermediate (b). The carbonyl reforms in the more stable amide (c), generating the alkoxide (d) as the leaving group. Essentially, one acyl group substitutes for another.

For biosensing we are primarily interested in the use of PCL as a conveniently degradable platform for oxygen sensing by attaching an FDA-approved porphyrin, PdBMAP. The resulting product would allow for constant oxygen monitoring following subdermal tissue injection[11]. The utilization of PdBMAP-functionalized PCL could plausibly provide verification that the parent chain was indeed aminolysed. However, this conclusion is unsatisfying as we are unable to actually confirm covalent modification via direct analytical confirmation. We rather see it through the effects expected from 'successful' attachment of this additional compound.



Scheme 1: PCL reacting with HMD.

Unsurprisingly, a long history of attempts at chemical attachment to PCL exists in which a variety of techniques have been utilized. In this work, we initially attempted to use both ^{1}H NMR and ATR-FTIR as a means of verifying the success or failure of the reaction mechanism shown in Scheme 1. These techniques made it clear that the molecular weight of the 80,000 M_n PCL undergoing amine modification was too high to allow for unambiguous amine detection. To combat this issue, we first had to reduce the molecular weight of PCL being activated via amine modification. Shifting from the use of 80,000 M_n to 10,000 M_n PCL created products of polymer scission that were much less rare and thus detectable by NMR. Even under these circumstances, it was difficult to isolate a single peak of one-dimensional proton NMR that conclusively demonstrated amide attachment. To obtain additional information from the NMR spectra, 2D experiments were run—specifically, Heteronuclear Multiple Bond Correlation Spectroscopy (HMBC)[12]. Via technique, direct aminolysis of 10,000 M_n PCL was successfully confirmed, simultaneously establishing a proven, more reliable pathway for chemical modification. Additionally, amide quantification was also demonstrated via XPS.

This amine-attached end group provided a substrate enabling the subsequent attachment of PdBMAP utilizing carbodiimide chemistry to link this oxygen-sensitive molecule to the PCL main chain which was then verified using UV-VIS[13]. Utilizing a purpose-built specific testing tank, this attachment was followed by an examination of its oxygen-sensing capability in electrospun form contrasted to so-called "guest host" blends of PdBMAP in a polymeric matrix. These results showed that – as expected – chemical attachment resulted in definitive improvement in sensor lifetime and sensing ability.

Material and methods

1) *Method 1*[13, 14]

Electrospun PCL (80,000 M_n; SIGMA-ALDRICH, 440744) was soaked in ethanol overnight and then was that immersed in a 10% w/w solution of HMD in IPA at 50 ^{O}C for 6h. The resulting PCL was rinsed with distilled water and left to dry.

2) Method 2[15]

Two separate 1:1 solutions of dioxane and IPA were created. One was used to dissolve PCL while immersed in a 40°C water bath while the other was utilized to dissolve HMD. These two solutions were combined forming a 5% w/w final HMD concentration heated for an additional hour while undergoing vigorous stirring at 60 rpm. The PCL was removed from the water bath and cold ethanol added via Pasteur pipette to precipitate the potentially functionalized polymer chain from solution. This was followed by a cold washing in DI water followed by filtration and drying in air.

3) Method 3

One gram of 10,000 M_n PCL (SIGMA-ALDRICH, 440752; 10,000 MW) and 10 mL of tetrahydrofuran (SIGMA-ALDRICH, 401757), were placed in an Erlenmeyer flask (250 mL) inside a glove box under N_2. Using a stirring hotplate (VWR, 12305-480), the flask was continuously agitated while being heated to 40°C for the duration of the chemical attachment process. In a separate Falcon centrifuge tube (Fisherbrand, 352196), 0.1 g of hexamethylenediamine (HMD; SIGMA-ALDRICH, H11696) was also dissolved in 5 mL of tetrahydrofuran. Next, 0.17 g of sodium hydride 60% dispersion mineral dispersion (SIGMA-ALDRICH, 452912) was weighed out and placed in 50 mL of hexane (MILLIPORE SIGMA, HX0291-1) within an Erlenmeyer flask for ~10 min. The supernatant containing the original mineral oil carrier was then removed via Pasteur pipet (Fisherbrand, 13-678-6A) and the pure NaH (0.1 g) remaining was dissolved in 5 mL of THF within a separate vortex tube. NaH solution (0.5 mL) was introduced into the HMD solution (2.35 mL) in a third vortex tube which allowed the resulting H_2 (g) to be freely released. This new mixture (85 μL) was then added to the Erlenmeyer flask containing both PCL and THF and left to stir for 1 h at 40°C. Approximately 50-100 mL of cold (4°C) ethanol was placed within a ~125 ml glass jar. A Pasteur pipet was used to transfer the entire contents of the Erlenmeyer flask into the ethanol-containing jar that was then left to stir for ~20 min. The final solution product was removed from the glove box and filtered (Fisherbrand, 09-795G), washed with DI water and left in a fume hood overnight to dry.

4) Tracer Dye Verification Via Rhodamine

Within a nitrogen-containing glove box, PCL (0.5 g) that had undergone amine attachment was dissolved in 5 mL THF stirring at 40°C within a glass vial on a stirring hot plate. In an Erlenmeyer flask, NaH (0.17 g) was left to sit in hexane (~50 mL) for 10 min to remove the protective mineral oil as before. The oil-containing supernatant was again removed using a Pasteur pipet. The pure NaH (1 g) was dissolved in THF (5 mL) within a vortex tube. Approximated 120 μL of this mixture was then added to the glass vial containing PCL and THF. In a separate vial, 95.80 mg of Rhodamine B (ACROS ORGANICS, 29657) was mixed with 2 mL ethanol and 1 mL of THF. This rhodamine dye solution was then added to the vial containing PCL. This was covered with aluminum foil and left to stir for 1 hr. The resulting PCL was then separated from the rest of the mixture via gravity filtration employing repeated ethanol washing (3 x 50 mL) on coarse filter paper (Fisherbrand, 09-795G). The filter paper was left to further dry overnight within a fume hood in a dark room.

5) Pd-BMAP—PCL Attachment Via NaH

Under a nitrogen atmosphere, 0.5 g PCL that had undergone the amine attachment procedure was dissolved in THF in 5 mL by stirring at ~40°C. This procedure took place in a glass vial on a hot plate under constant stirring. NaH (1 g) was dissolved in THF (5 mL) within a vortex tube. Approximately 120 μL of this mixture was added to the glass vial containing 270 μL of the PCL, THF, and Pd-BMAP solution. This was covered with aluminum foil and left to stir overnight. The next day, this mixture was precipitated into ethanol in a ~150 mL glass jar under continuous stirring for 20 min. Afterward, the PCL was separated from the rest of the mixture via gravity filtration employing additional ethanol washes (3 x 50 mL). The filter paper was left to further dry overnight in the fume hood of a darkened room while covered with aluminum foil to avoid light exposure. The vial was then placed in a vacuum overnight prior to analysis.

6) Pd-BMAP—PCL Attachment Via EDC chemistry

Approximately 0.125 g of the aminolysed PCL from Method 3 above was mixed with THF (2 mL) in a glass vial containing a stir bar and allowed to dissolve. In a separate vial, 50 mM EDC (SIGMA-ALDRICH, E7750) and 25 mM NHS (SIGMA-ALDRICH, 130672) solutions were added to dissolve in 2mL THF. This solution was added to the PCL vial containing 200 μL of a Pd-BMAP-COOH (Profusa Medical, Emeryville CA). This vial was then wrapped in aluminum foil and left to stir (~60 rpm; 40°C) overnight. The next day, the mixture was precipitated in ethanol (~5-10 mL) under continuous stirring and filtered via gravity along with additional ethanol washes (3 x 50 mL) followed by a 24 h soak in dimethyl sulfoxide (DMSO; Fisher Chemical, D128-1). The filter paper was left to further dry in the dark room fume hood covered with aluminum foil to avoid light exposure. The vial was then placed in a vacuum overnight prior to analysis.

7) UV-VIS Preparation

A stock solution of PCL (20 mg) dissolved in dioxane (10 g; SIGMA-ALDRICH, 360481) was created in a glass vial. This mixture was used to create five Pd-BMAP standards (0, 1, 5, 10, and 20 mM). The tabulated amounts of Pd-BMAP shown in Table 1 were added to each UV-VIS cuvette along with 600 μL of stock solution. As the absorbances resulting from this procedure were initially too high, their Pd-BMAP concentrations were reduced by 50%. The final Pd-BMAP potentially attached to PCL was prepared for concentration analysis by mixing 0.24 mg of the sample in question with dioxane (600 μL).

Pd-BMAP (mM)	Pd-BMAP (μL)
0	0
1	0.22
5	1.1
10	2.2
20	4.4

Table 1: Stock Solutions of Pd-BMAP.

8) NMR Preparation

In a small, disposable glass vial, amine-attached PCL was weighed out (~10-15 mg; Ohaus SPX622). Deuterated chloroform (1000 μL; CIL, DLM-7-PK) was pipetted (LabGenius, 100-1000 μL, YL5E106007) into the vial for mixing and the resulting solution transferred into NMR tubes (SIGMA-ALDRICH, Z272019) for testing. A Bruker Avance III 600 MHz NMR spectrometer operating at 600.18 MHz for ^{1}H and 150.93 for ^{13}C was used to collect high resolution 1D and 2D NMR spectra such as COSY, NOESY, HSQC, and HMBC NMR. The number of scans for ^{1}H NMR ranged from 16-2048. Additionally, the number of scans were 6 with the number of free induction decays (FIDs) set to 2048 for HMBC NMR. As for COSY, NOESY, and HSQC NMR the FID size was adjusted to 256. NOESY had a mixing time of 0.3 sec due to the size of the polymer chain; the rest of the NMR techniques employed had a mixing time of one second. The software used to set each run up was Topspin 4.0.9.

Results

1) *UV-VIS of Pd-BMAP-Attached PCL*

Method 5 attempting Pd-BMAP—PCL attachment via NaH activation resulted in a paper-like, fragile solid that was a uniform pastel green in appearance. UV-VIS analysis of this product did not result in a tangible, numerical concentration when compared to Method 6 via EDC chemistry. The appearance of this reaction product was a darker green than that of Method XX.

Pd-BMAP—PCL standards and conjugated PCL were both analyzed via Agilent Technologies Cary 5000 UV-Vis-NIR instrumentation. The EtOH-labeled sample in Figure XX represents the product of Method 6 without the DMSO soak. The DMSO-labeled sample is also Method 6 with the addition of an overnight soak to remove excess Pd-BMAP not chemically attached to the polymer chain. Pd-BMAP absorbs at 444 nm.

	Pd-BMAP mM
EtOH	4.45
DMSO Washed	0.61

Table 2: UV-VIS Pd-BMAP concentrations from calibration curve.

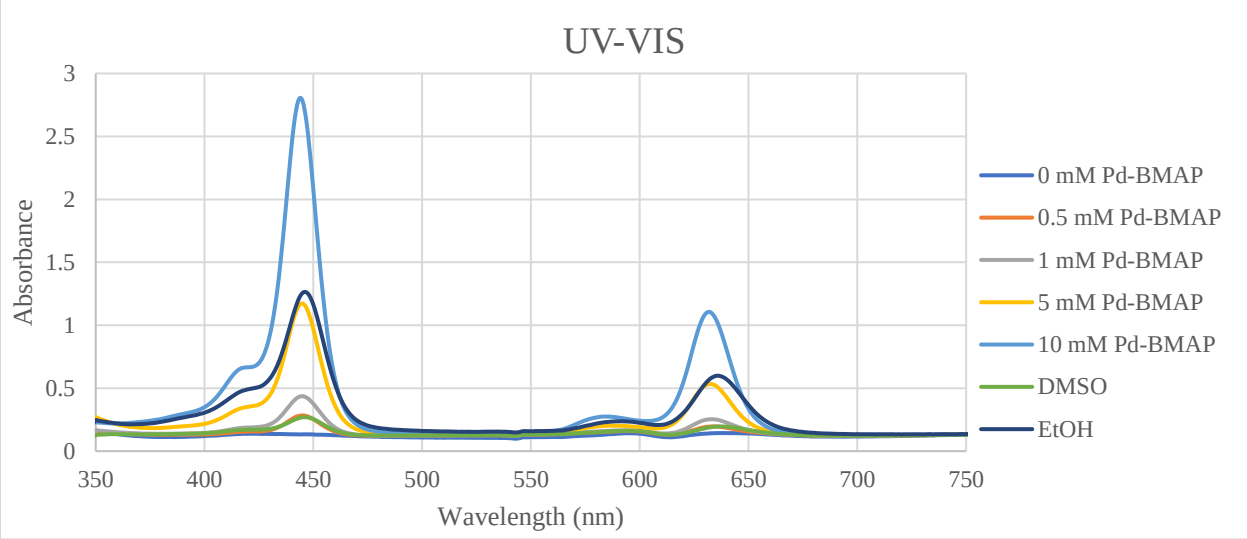


Figure 1: UV-VIS Spectra of Standards along attached PCL-Pd-BMAP.

2) ATR-FTIR

An Agilent Technologies Cary 630 FTIR with an ATR probe was also utilized. Eight background and sample scans were taken using a resolution of 2 over the range from 4000 to 800 cm^{-1}. Pure PCL— not exposed to HMD — was used as a baseline to create a replica spectrum of PCL chain containing nitrogen-bearing groups such as amines and amides. Previous studies supposedly confirmed the formation of aminolysed PCL via ATR-FTIR[16]. However, we could not confirm this output using these simulated spectra due to differences in either methodology or FTIR software configuration.

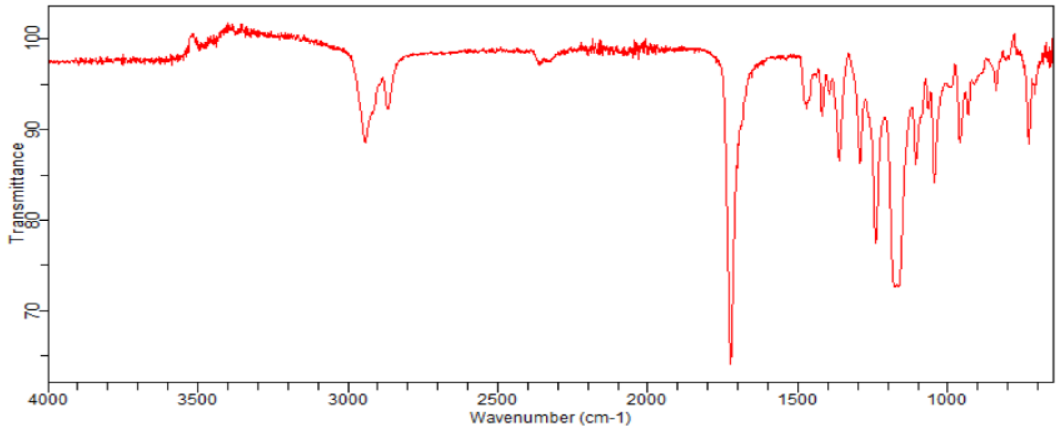


Figure 2: Pure PCL.

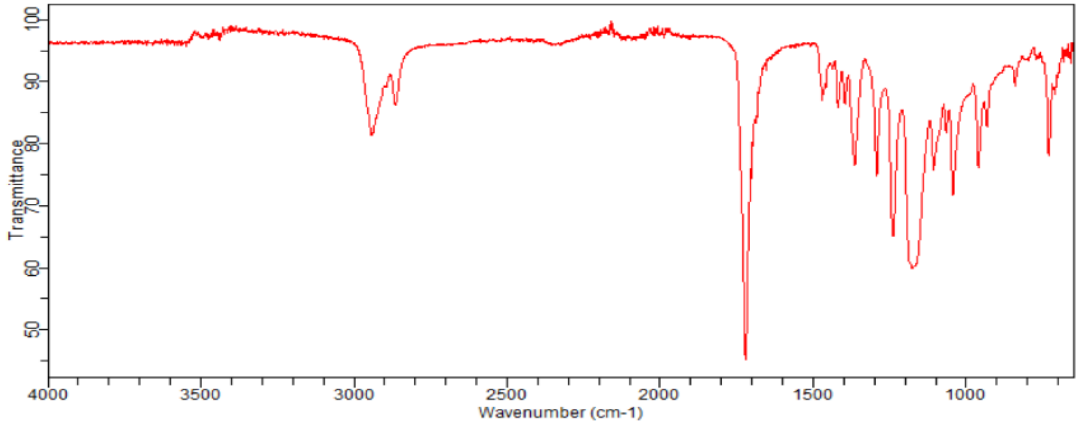


Figure 3: Simulated aminolysed PCL.

3) *NMR*

Below are the collected spectra showing both failed and successful NMR spectra of the various types.

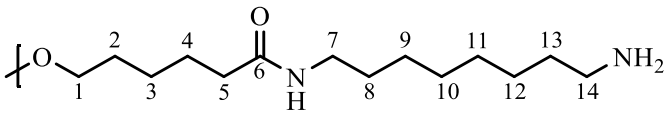


Figure 4: Final aminolysed PCL structure.

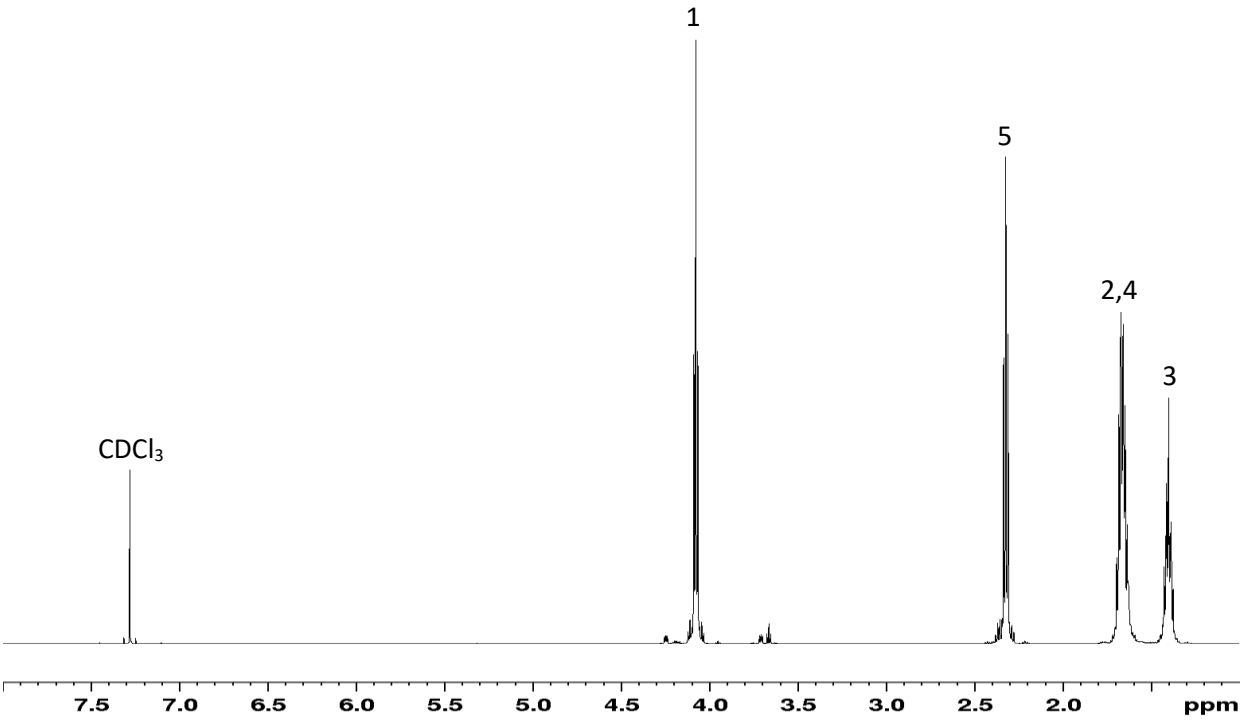


Figure 5: Pure PCL ^{1}H NMR.

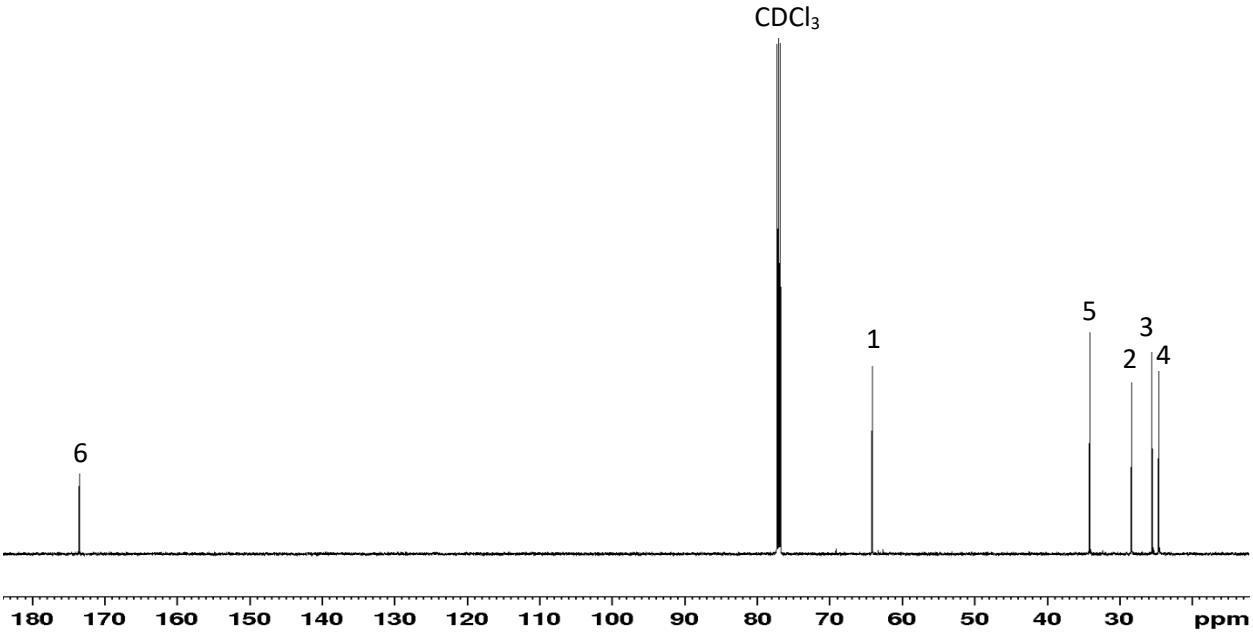


Figure 6: Pure PCL ^{13}C NMR.

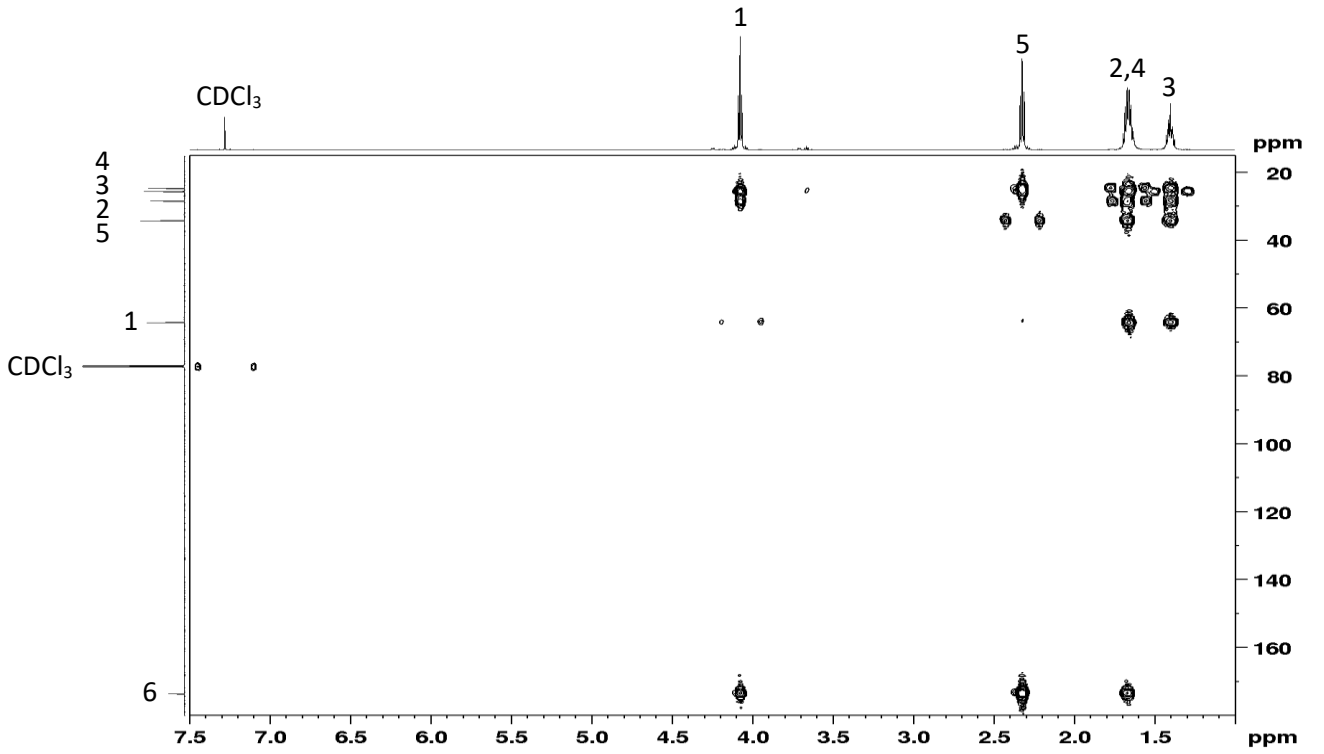


Figure 7: Pure PCL HMBC NMR.

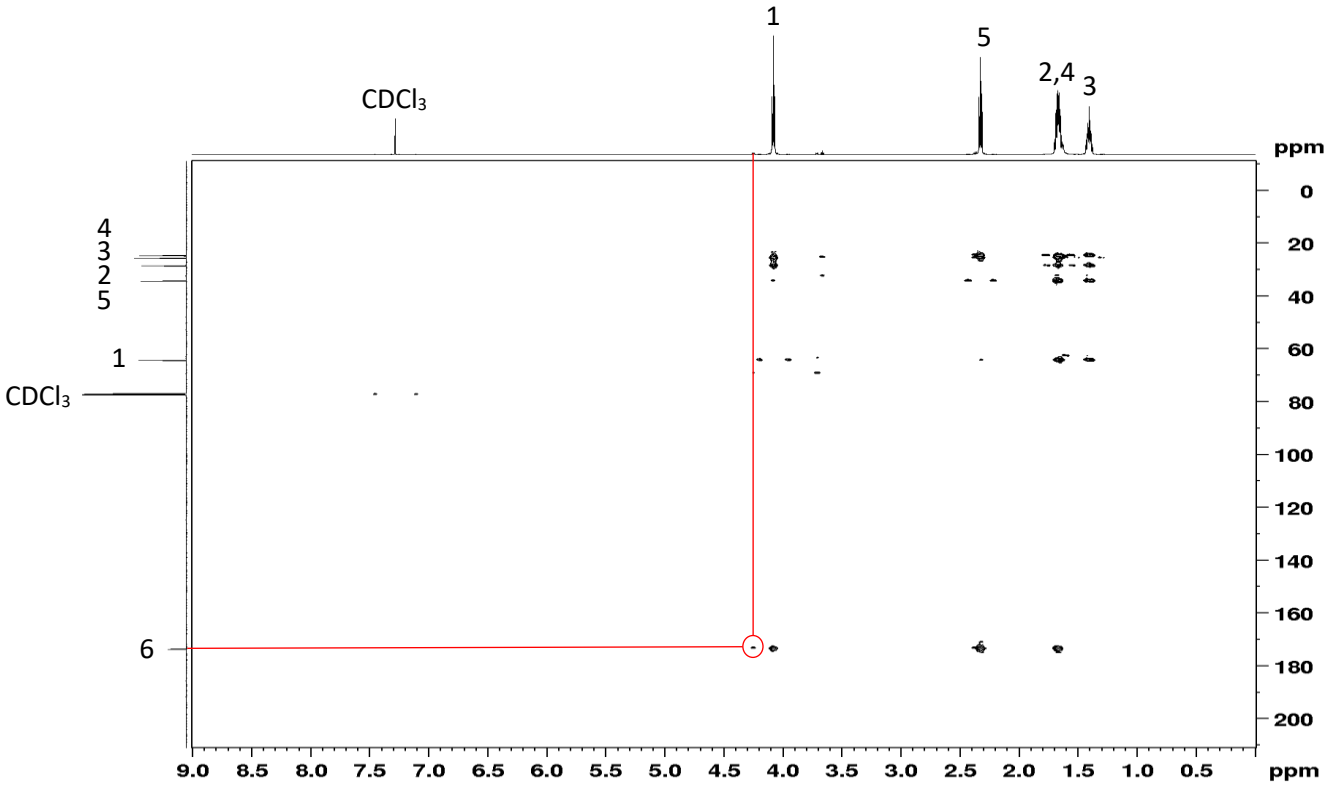


Figure 8: Aminolysed PCL HMBC NMR (Method 3; 10,000 M_n).

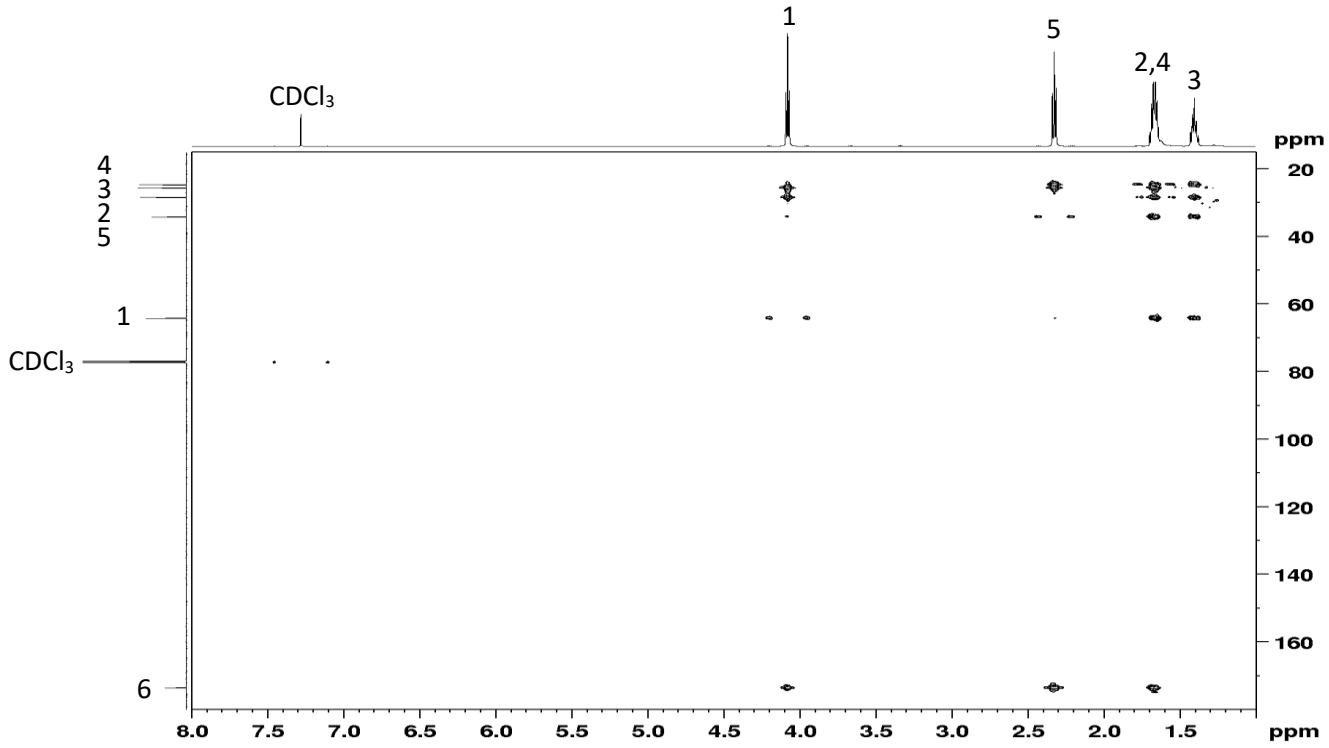


Figure 9: Aminolysed PCL HMBC NMR (Method 2; 10,000 M_n).

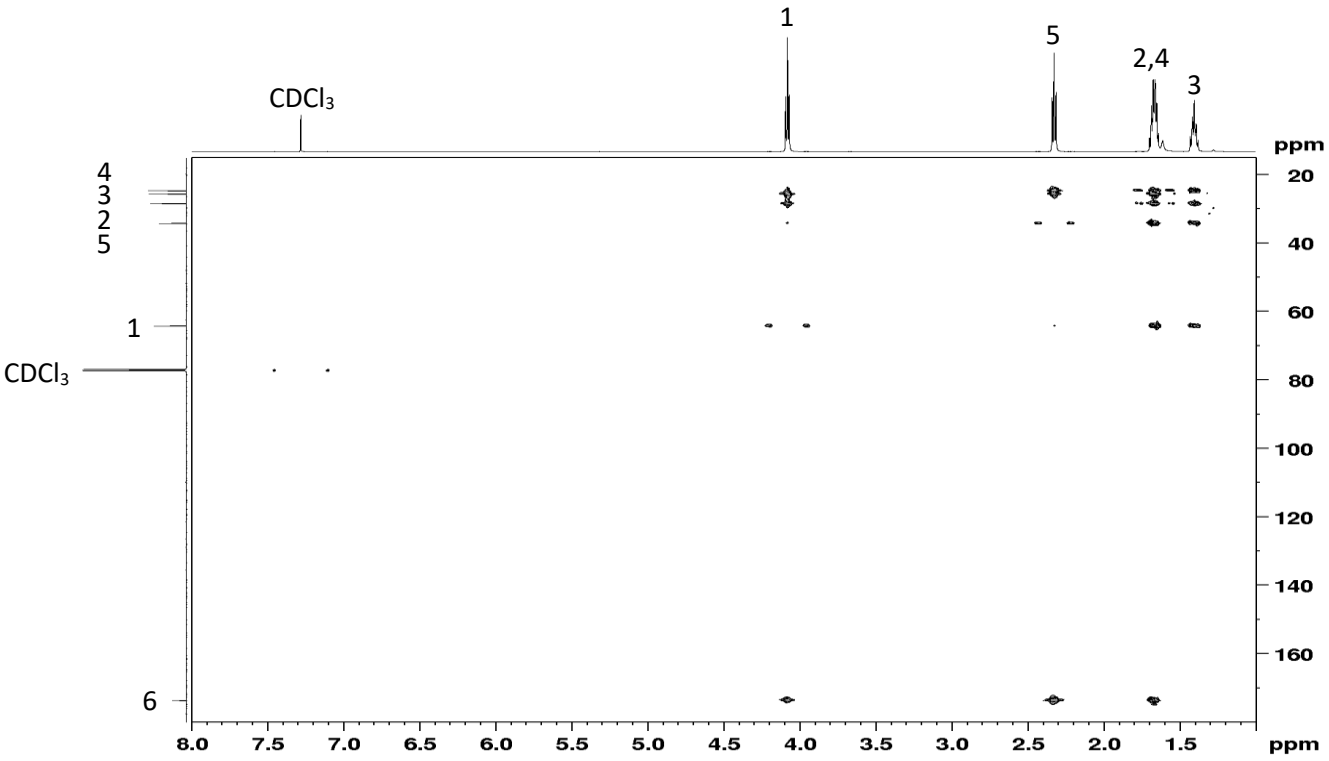


Figure 10: Aminolysed PCL HMBC NMR (Method 1; 40 ^{O}C, 1 hr; 80,000 M_n).

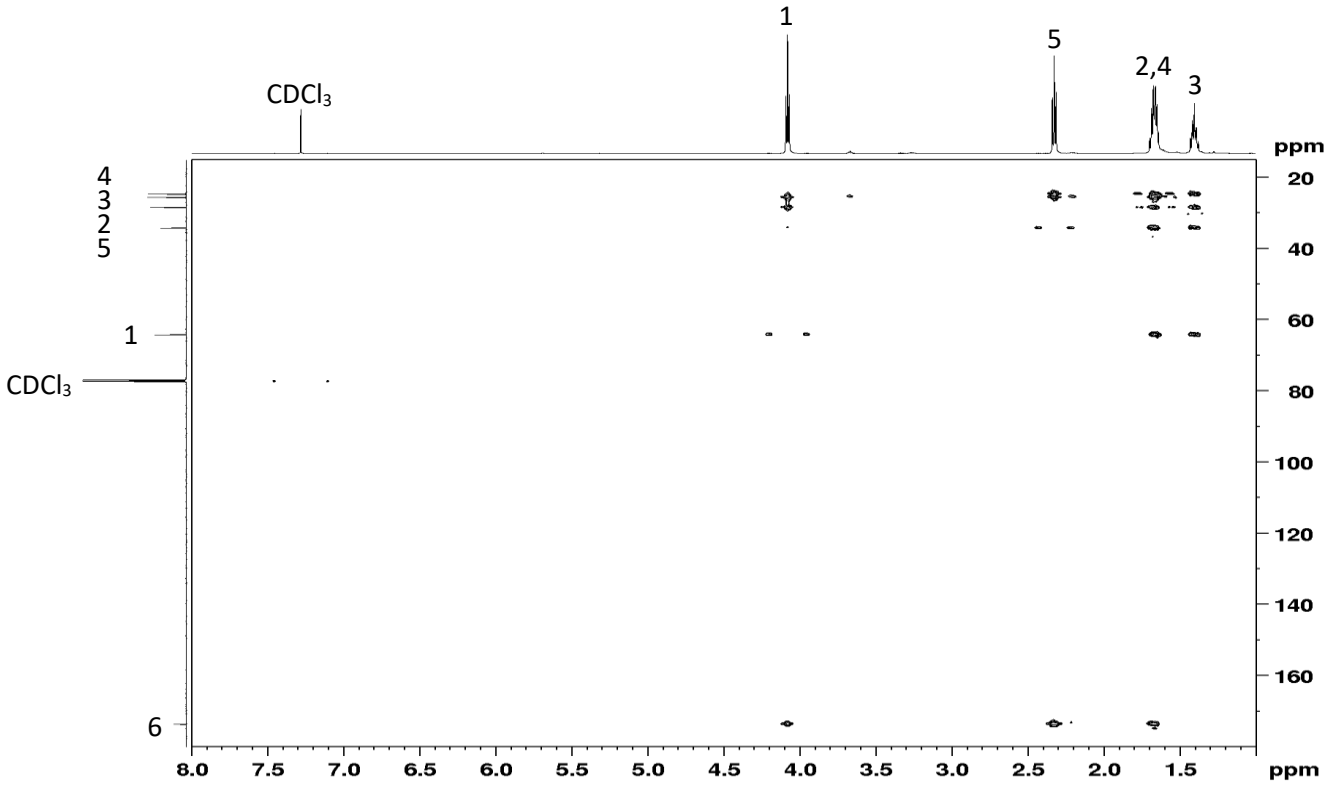


Figure 11: Aminolysed PCL HMBC NMR (Method 1 ;50 ^{O}C, 6 hrs; 80,000 M_n).

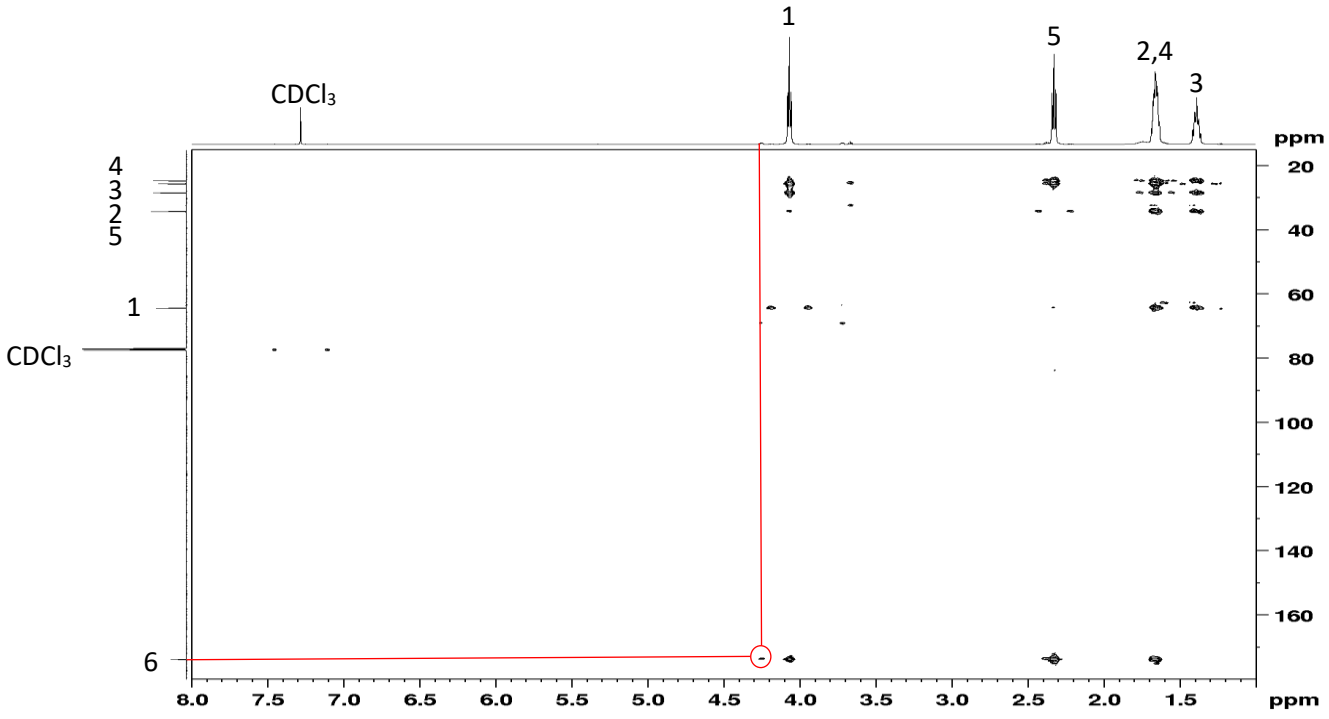


Figure 12: Aminolysed PCL HMBC NMR (Method 1; 50 ^{O}C, 6 hrs; 10,000 M_n).

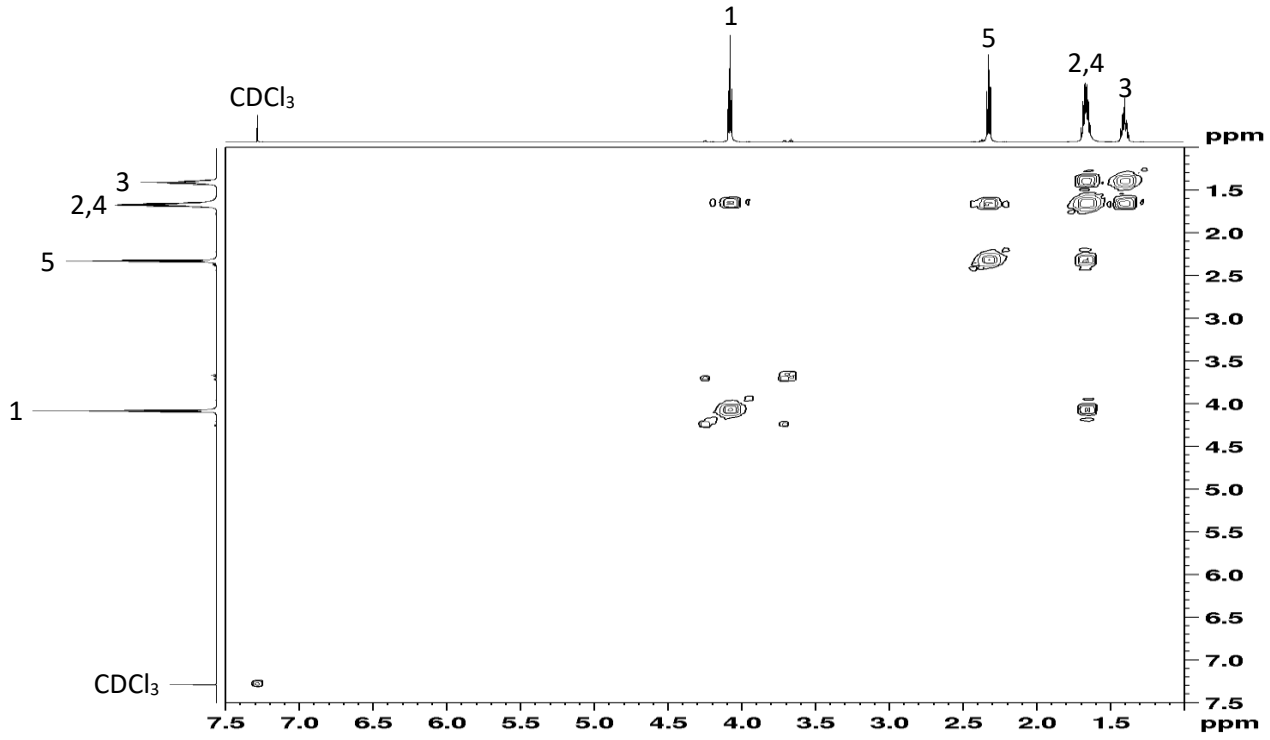


Figure 13: Aminolysed PCL COSY NMR (Method 3; 10,000 M_n).

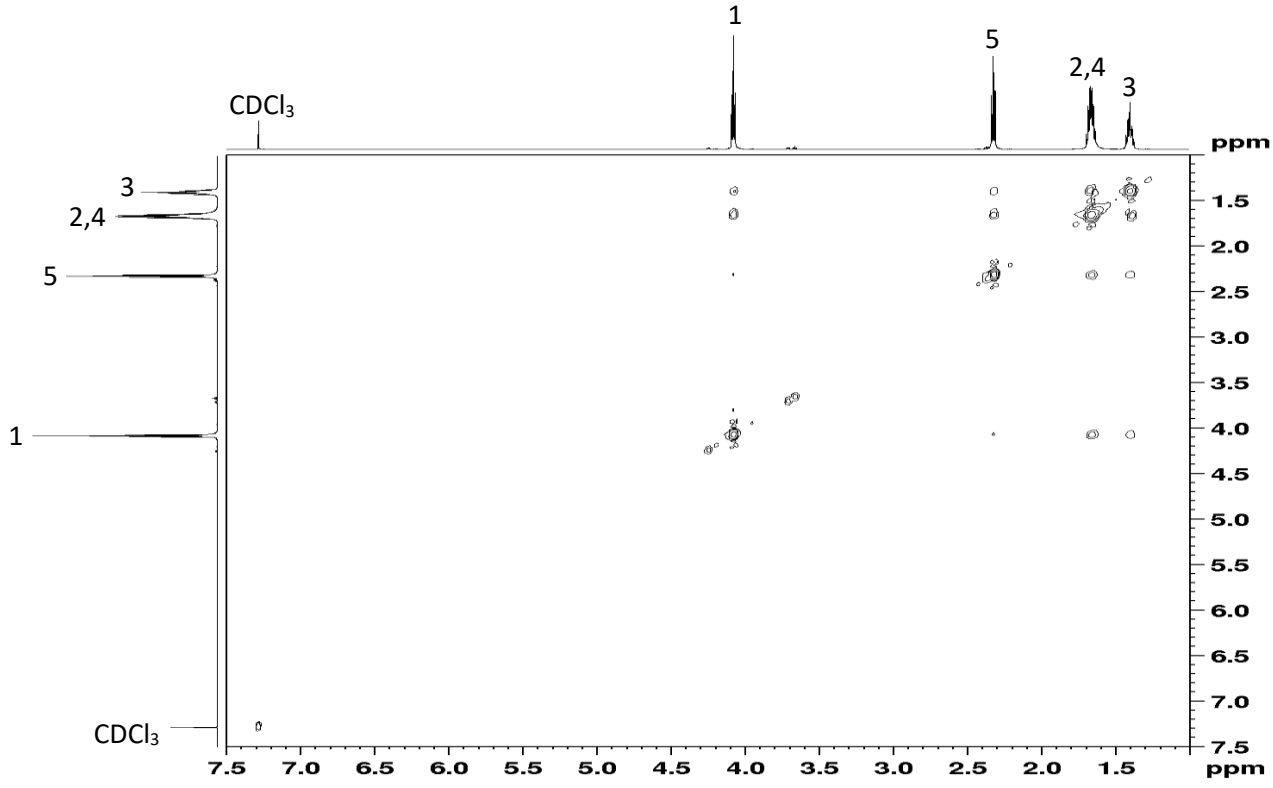


Figure 14: Aminolysed PCL NOESY NMR (Method 3; 10,000 M_n).

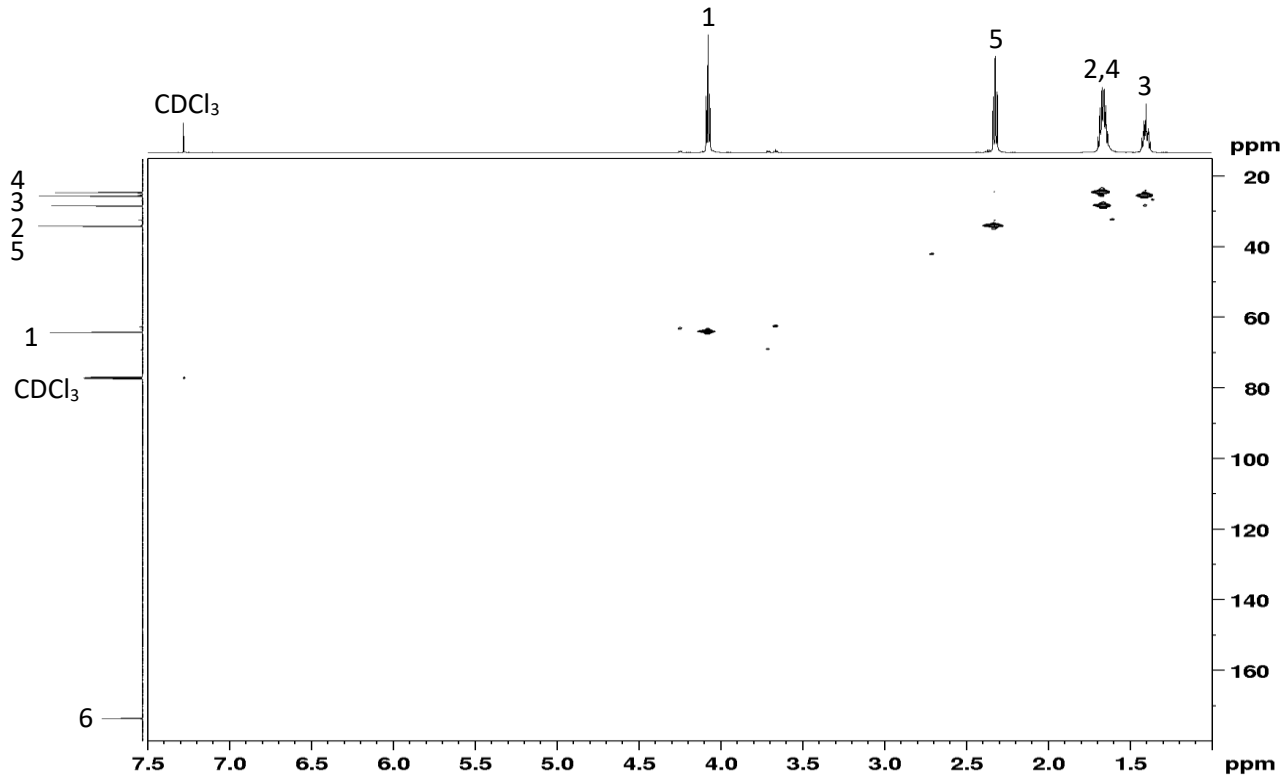


Figure 15: Aminolysed PCL HSQC NMR (Method 3; 10,000 M_n).

4) *XPS*

XPS measurements were conducted on a Kratos Ultra XPS utilizing monochromatic Al K-alpha x-rayssource (operated at 10 mA emission and 12kV). Survey spectra were collected using a step size of 1 eV and a 100 ms dwell time. High resolution spectra were obtained at 0.1 eV and 20 pass energy at a 300 ms dwell time for all three elements — C 1s, N 1s, and O 1s. Carbon and oxygen were collected using 4 scans while nitrogen was collected using 16 scans. As-received PCL displayed the expected atomic composition of 26.16% oxygen and 73.84% carbon. In contrast, for Method 3 aminolysed PCL, XPS data revealed a 16.47% oxygen, 80.14% carbon, and 3.39% nitrogen atomic composition corresponding to the anticipated 1:37 ratio of amide-bonded PCL to as-received PCL.

	Peak Energy (eV)	Peak Area	Concentration (at. %)
Oxygen	529.80	6759.91	16.47
Nitrogen	397.60	782.124	3.39
Carbon	282.70	9610.27	80.14

Table 3: XPS raw data of aminolysed PCL

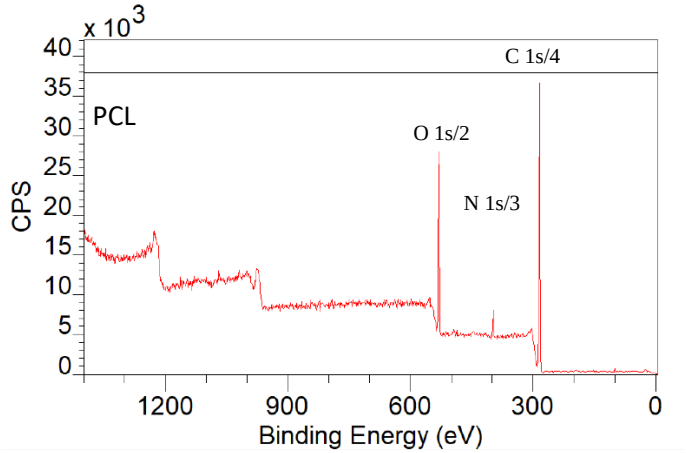


Figure 15: Survey data from Method 3 aminolysed PCL.

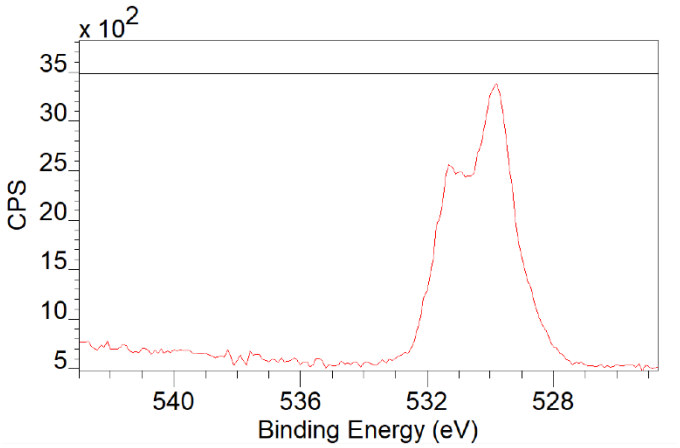


Figure 16: High-resolution oxygen spectrum taken from Method 3 aminolysed PCL.

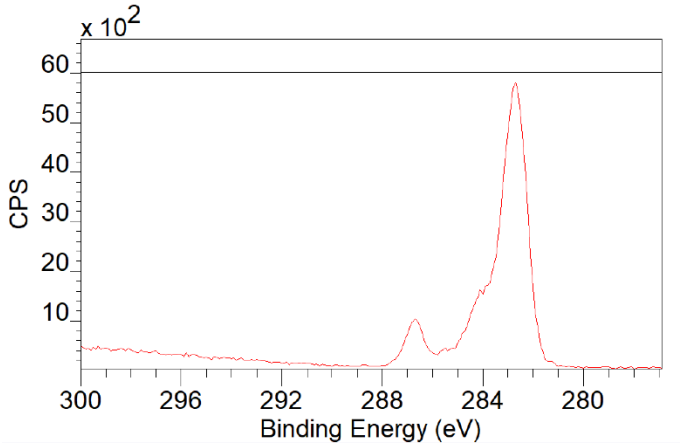


Figure 17: High-resolution nitrogen spectrum taken from Method 3 aminolysed PCL.

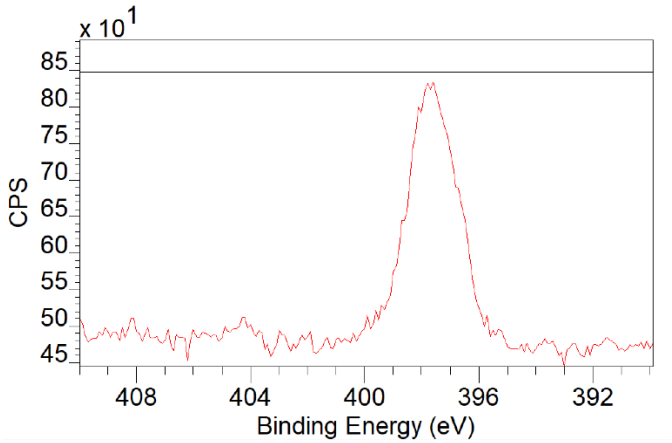


Figure 18: High-resolution carbon spectrum taken from Method 3 aminolysed PCL.

5) *Rhodamine*

The pink, rhodamine pigment was not uniform in the product and came out as a light tint with white patches peeking through. The visual failure put a halt to any additional testing.

Discussion

It was established that verifying the successful attachment of a functional group, in this case an amine, to a high polymer using NMR is far from ideal. While NMR and ATR-FTIR are individually very useful methods, they proved unsuited to the task of examining products relying on the limited scission of

initially high (80,000 M_n) molecular weight polymers. Modification at a scale of 80,000:1 PCL were undetectable by all methods utilized here. Additionally, chemical modification of these high molecular weight PCL chains with excess HMD still could not definitively prove that that amine groups were being chemically attached to the parent polymer chain.

In contrast, much more positive results were achieved by lowering the initial molecular weight from 80,000 to 10,000 M_n and observing the reaction results both spectrally and chemically. Examined using 2D NMR, the aminolysis of 10,000 M_n PCL yielded promising results. Based on XXXX, these established that successful amination of the polymer chain (Figures 8 and 12) had indeed occurred.

In contrast, 1D NMR was clearly not sufficient to confirm successful aminolysis[17]. Two-dimensional NMR via homonuclear correlation spectroscopy, space correlation spectroscopy, and heteronuclear correlation spectroscopy were explored. Homonuclear spectrums are collected through J-coupling of the same nuclei type. An example of such an analysis is Correlation Spectroscopy (COSY), which was the initial method employed. These spectrums further confirm the structure of the repeat unit of the polymer chain. We see there is a correlation between 1.68 ppm and 1.40 ppm that corresponds protons from carbons 2 and 4—as they share the same chemical shift—with carbon 3, respectively. This initial run of COSY did not provide useful information. After running HMBC NMR with foreknowledge of where to look for the amide proton, we still could not obtain useful information from the COSY spectrum. This method cross-peaks adjacent protons and expecting additional information is a stretch due to the amide being covalently attached to a carbonyl which itself possess no hydrogens to cross peak with.

Finally, Nuclear Overhauser effect spectroscopy (NOESY) was initiated with the expectation of physical proximity of the amide and any major parent chain nuclei being detected via cross-relations[18]. Utilizing this type of NMR, we expected to correlate the hydrogens on carbon 5 with those on carbon 3 (see Figure 43) located at 2.32 ppm and 1.40 ppm, respectively. The hope behind the implementation of this technique was to correlate the protons of carbon 5 with those of the the newly added amide group. However, we found that this was not the case due to either to the motion of the polymer or a lack of overall

sensitivity[19]. While COSY and NOESY are similar, they differ in what type of instrumental detection occurs—bond coupling versus spatial environment, respectively.

Finally, heteronuclear correlations were also explored. This type of spectroscopy couples nuclei of varying types[20]. Heteronuclear single-quantum correlation spectroscopy (HSQC) evaluates the two nuclei types at only one bond length of separation, insufficient for these functional polymer chains as it is another method only for the parent polymer chain structure confirmation. For example, such a method focuses on one-bond couplings and identifies direct carbon and/or nitrogen bonding with protons. However, the scarcity of these end groups meant that the amide's nitrogen and hydrogen could not be detected in the resulting spectrum. We do see parental chain correlation, however. For example, there is a correlation between carbon 5 and its protons at 34.4 ppm and 2.33 ppm, respectively, which corresponds to the expected chemical structure (Figure 4).

The alteration required was not verified through one-bond coupling but detection could be achieved through multiple bonds, a sensitivity provided using heteronuclear multiple-bond correlation spectroscopy (HMBC). HMBC has previously been used to characterize homopolymers, copolymers, and terpolymers[21]. This technique provides what is known as an inverse detection method which has high sensitivity to protons in solution and ignores inactive nuclei which would otherwise be reported—this can also be said to apply to HSQC NMR[22]. Furthermore, the end group –NH possesses a chemical shift over a large range that unfortunately balances out the advantages of HMBC NMR analysis. Amine and amide compounds have ppm ranges of 1-5 and 5-6.5, respectively. However, identifying the amine end group is far from ideal due to the fact that the HMD reactant lacks any and all NMR presence. On the other hand, the amide is more realistic to pinpoint as it is the bond that is directly attached via a covalent bond to the PCL parent chain.

This technique, HMBC, employs multiple-bond coupling to map cross-peaks between a carbon and specific protons located as much as two or three bonds down a given chemical chain. While nitrogen cannot be directly detected via NMR, its attached hydrogens are thus theoretically able to be identified. The chemically-attached primary amide on the PCL chain is not easily distinguishable from the rest of the

polymer's hydrogens. This is due to the initial high molecular weight of the polymer chain and also to how vigorously it these chains were cleaved by HMD exposure. Once the PCL M_n was theoretically reduced to ~one-eight of its original length, we were able to use HMBC to assign the amide peak, which was already present in ^{1}H NMR, but disregarded there as noise. A clear correlation exists between the protons at 4.25 ppm and the carbons at 173.6 ppm. The carbon peak corresponds to the carbonyl carbon—carbon 6 in Figure 4. The proton, however, does not correlate to any parent chain hydrogen. As HMBC NMR cannot detect any single bond correlations and is useful only for assigning carbons that have no proton attachment, we conclude that carbon 6 cross peaks with the hydrogens of carbon 7. The 4.25 ppm proton is two bonds away from the carbonyl carbon and must be that which we have attempted to definitively identify as being the amide proton; this successfully confirms aminolysis. It should also be noted that within the NMR figures, there are artifacts within the HMBC spectra in origin of the ^{13}C satellite[23]. Having this in mind is what allowed us to distinguish possible amide cross peaks from additional new peaks.

Interestingly, we see what we have identified as aminolysis via HMBC NMR is also observed following Method 1 when the molecular weight of the reactant PCL is decreased from 80,000 to 10,000 M_n via exposure to HMD (Figure 12). This conclusion was influenced by a literature study that had been optimized in the laboratory to achieve tangible aminolysis data[14]. Reaction duration was initially increased from 10 minutes to 6 hours but that was likely not the determining factor for enabling observation of covalent amine attachment via HMBC. Rather, it was the reduced M_n that made these results more clearly observable.

Additional points of confirmation come following both XPS spectral analysis and Pd-BMAP attachment. XPS was able to verify the presence of a nitrogen-containing species following Method 3. The presence of this moiety is slightly questionable when examining the obtained spectra in comparison to literature values[24]. Our data shows a binding energy of 397.5 eV while the literature values range from 399-401 eV for amides, amines, and other nitrogen species[25, 26]. Amines (399.5 eV) have a higher binding energy than do amides (398.4 eV)[27]. When examining our collected data, the nitrogen-bearing moiety has a low

binding energy—lower than even an amide. Realistically, we should be able to see two origins for nitrogen in our aminolysed structure (Figure 4). However, we cannot say which species we do detect but that we do detect a functional, nitrogen-bearing group.

In addition to the goal of proving modification, we went a step further to attach specific functional groups by activating the diamine reagent and utilizing carbodiimide chemistry to attach Pd-BMAP. By creating a linear fit of the standards (UV-VIS Preparation from the Materials and Methods section), the coefficient of determination was determined to be 0.9851 (Figure 1) that allowed for high quality measurement of the PCL-attached Pd-BMAP. From the concentrations listed in Table 2, we see the difference between chemically attached Pd-BMAP and that which is simply physically mixed with the PCL. The targeted concentration of the attached porphyrin was 1 mM; while our current method might require improved chemistry for better determination of concentration, we can conclude covalent attachment indeed took place. This addon could only have occurred if an amine is present for a reaction that is connected to the polymer chain, PCL. Therefore, we can conclude the starting material of Pd-BMAP attachment was indeed aminolysed. Examination of the as-synthesized compound before and after this step allowed a comparison that might be indirect but effectively establishes that this compound was indeed aminolysed following the intermediate step.

Other methods such as ATR-FTIR and the rhodamine tracer dye were not at all successful in verifying amine attachment. If anything, they suggest that it does not occur. Having said that, the issue derives not from the method by which PCL was aminolysed, but rather the tests conducted to confirm HMD attachment. This issue is again due to the low concentration of the amines attached to the parent chain for ATR-FTIR detection[28]. And while certainly other studies have showed effective use of rhodamine b isothiocyanate and ATR-FTIR for amine group labeling, that was not the case in our experiments[29, 30].

Conclusion

We have established a method for direct amination of PCL. While prior work suggests that other methods can aminate PCL, we were unable to detect the relevant chemical bonds using our techniques

following our attempts to reproduce these methods. While these methods were apparently able to introduce amine groups into the polymer, covalent attachment of the amine may not have actually occurred. We developed an improved method for amination and have also conclusively shown the correct chemical attachment of the amine group. Possessing this basic framework, covalent attachment was conclusive through HMBC NMR of the attached amide allowing the polymer chain to be functional. The groundwork for verification was done using XPS, and subsequent chemical attachment of Pd-BMAP via EDC chemistry, utilizing the produced amine group.

Future work

Even though the identification and verification process of aminolysed PCL was successful, additional measures that could be taken to further prove covalent attachment. Nitrogen 15 labelling can be conducted to guarantee accurate and reliable proton NMR identification without the need for additional 2D NMR analysis. For more cost-effective exploration, an even lower M_n of PCL should be reacted with HMD via same methods and verification reported using NMR and XPS. Mass spectroscopy could be implemented to identify the polymer chain repeat unit alongside additional elemental peaks corresponding to the attached amine end groups. Finally, sensitive chemical reactions known to quantify low amine concentrations should be further explored.

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